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The Potent Platelet Inhibitory Effects of S-Nitrosated Albumin Coating of Artificial Surfaces

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OBJECTIVES We studied the antithrombotic effect of coating glass, collagen and metal stent surfaces with bovine serum albumin (BSA) covalently modified to carry S-NO functional groups denoted (pS-NO-BSA).

METHODS Video-enhanced light microscopy was used to visualize canine blood platelet adhesion and aggregation in a parallel plate glass chamber. Platelet adhesion was observed for 60 min on glass, glass coated with BSA, glass coated with pS-NO-BSA, collagen I (CO) surface, CO coated with BSA and CO coated with pS-NO-BSA. We also coated Palmaz-Shatz (P-S) stents with pS-NO-BSA. Coated and uncoated stents were then immersed in porcine platelet-rich plasma for two min and the platelet cyclic GMP level was measured. In six anesthetized pigs, coated and uncoated stents were placed in the carotid arteries and [^{111}In]-labeled platelets were circulated for 2 h. The stented arteries were then removed and placed in a gamma well counter.

RESULTS There was significantly less platelet attachment, adhesion and aggregation on the pS-NO-BSA coated surfaces compared with the BSA coated and uncoated surfaces. The pS-NO-BSA coating increased the platelet cGMP levels to 5.9 ± 0.7 pmoles/10^8 platelets compared with 2.7 ± 0.9 pmoles/10^8 platelets for control ($p < 0.01$). The average gamma ray count from [^{111}In]-labeled platelets that attached to the coated stents was 90,000 ± 42,000/min and 435,000 ± 290,000/min for the uncoated stents ($p < 0.01$).

CONCLUSIONS The pS-NO-BSA coating of thrombogenic surfaces reduces platelet adhesion and aggregation, possibly by increasing the platelet cGMP. This inhibitory effect appears to be a consequence of the direct antiplatelet actions of NO combined with the antiadhesive properties of albumin. (J Am Coll Cardiol 1999;33:1408–14) © 1999 by the American College of Cardiology

Endothelium-derived relaxing factor (EDRF), which is closely related to nitric oxide (NO), is one of the primary antithrombotic factors produced by the vascular endothelium (1). NO (2,3) and NO-donating compounds, such as nitroglycerin (4) and sodium nitroprusside (5) given intravenously, were found to inhibit platelet interactions with damaged and stenosed canine coronary arteries. Endothelial NO inhibits both platelet adhesion and platelet aggregation. Nitric oxide also relaxes vascular smooth muscle cells (2) and inhibits vascular smooth muscle cell proliferation (6). Removal of the endothelium by plaque rupture or acute vascular damage is a potent stimulus for thrombosis and neointimal hyperplasia which are believed to be key events in atherogenesis and restenosis after angioplasty.

Albumin is often used for coating vascular grafts to passivate surfaces in contact with blood and thus minimize surface-induced platelet activation (7,8). Albumin reduces both the number of adherent platelets and the extent of platelet activation on the albumin-adsorbed surface (7). We have previously shown that serum albumin can be modified to bear a covalently linked S-NO functional group that manifests nitrovasodilation and platelet inhibitory properties (9). More recently, we chemically modified bovine serum albumin (10) so that the molar ratio of S-NO to albumin is greater than 1:1. We have shown that this produces a polynitrosated albumin (pS-NO-BSA) which can be applied locally to foreign surfaces or to severely damaged arterial walls to make them less thrombogenic (11,12). We have also shown that pS-NO-BSA can also be applied acutely for ten min to angioplastied rabbit femoral arteries and that this acute local coating significantly reduces intimal hyperplasia observed 21 days later (10). Thus, we postulated that pS-NO-BSA might be used to coat glass,

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Abbreviations and Acronyms

BSA	=	bovine serum albumin
pS-NO-BSA	=	BSA covalently modified to carry multiple S-NO functional groups
EDRF	=	endothelium-derived relaxing factor
NO	=	nitric oxide
P-S	=	Palmaz-Shatz
PRP	=	platelet rich plasma
TCA	=	trichloroacetic acid

collagen or P-S stent surfaces and render them less thrombogenic. To test this hypothesis, we chose to: 1) study the extent of platelet adhesion, spreading and aggregation on uncoated, BSA-coated and pS-NO-BSA-coated glass and collagen surfaces and 2) test whether or not the coating would adhere to a metallic stent surface and thereby reduce platelet activation and attachment to the stent both in vitro and in situ.

METHODS

Parallel plate chamber experiments. We used an in vitro parallel plate chamber fitted to an inverted video-enhanced microscope which permitted us to visualize and video record the activation of individual platelets on glass and collagen surfaces (13,14). The parallel-plate chamber was made by producing a gap (10 mm × 20 mm × 120 μm) between a 20 × 20 mm glass cover slip and a 24 × 50 mm glass cover slip separated by 120 μm thick parafilm (Fig. 1). The 24 × 50 mm glass cover slip was coated with the material to be exposed to the platelets. Video-enhanced light microscopy with digital image processing was used to examine platelet interactions with collagen, collagen coated with BSA and collagen coated with pS-NO-BSA. The collagen reagent was obtained from Chrono-log Co. (Harvertown, Pennsylvania). The bovine serum albumin (BSA) was obtained from Sigma Chemical Co. (St. Louis, Missouri). The pS-NO-BSA was synthesized in our laboratory as described below.

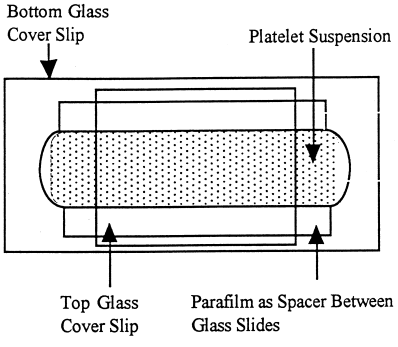

Figure 1. Parallel plate chamber used to visualize platelet attachment and spread over coated glass surfaces using video-enhanced light microscopy. The parallel-plate chamber was made by producing a gap between a (20 × 20 mm) glass cover slip placed on top of a (24 × 50 mm) glass cover slip separated by parafilm.

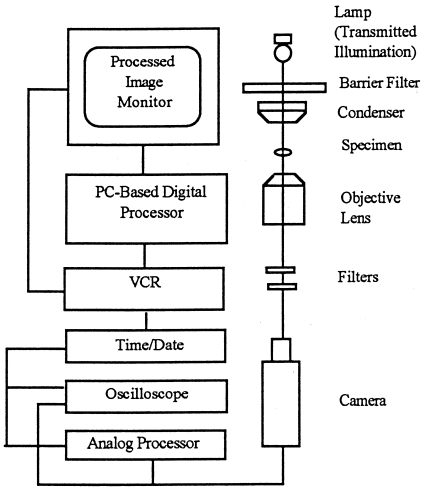

Figure 2. Schematic drawing of video-enhanced light microscopy. A modified inverted Nikon Diaphot equipped with Nomarski optics. A Dage-MTI (Model 65, MK III Series, Dage-MTI, Inc., Michigan City, Indiana) was attached to the microscope to produce a video signal of the microscope image. The video signal was processed by a CVI Model 302-2 Sync Stripper (Colorado Video, Inc., Boulder, Colorado) and CVI Model 604 Video Processor (Colorado Video, Inc., Boulder, Colorado). The final signal was recorded on a Panasonic NV-9240XD 3/4″ video recorder (Panasonic Industrial Co., Secaucus, New Jersey). The video image was processed by Image-1 (Universal Imaging Corporation, Media, Pennsylvania) hardware and software which was installed in a personal computer.

Video-enhanced light microscopy. We performed light microscopy using a modified inverted Nikon Diaphot equipped with Differential Interference Contrast (DIC) optics (Fig. 2). A Dage-MTI (Model 65, MK III Series, Dage-MTI, Inc., Michigan City, Indiana) was attached to the microscope to produce a video signal of the microscope image. The video signal was processed by a CVI Model 302-2 Sync Stripper (Colorado Video, Inc., Boulder, Colorado) and CVI Model 604 Video Processor (Colorado Video, Inc., Boulder, Colorado). The time and date were added to the processed signal by a Panasonic WJ-810 time/date generator (Panasonic Industrial Co., Secaucus, New Jersey). The final signal was recorded on a Panasonic NV-9240XD 3/4″ video recorder (Panasonic Industrial Co., Secaucus, New Jersey). For computer image analysis, the video signal was processed by a FA-210 Digital Time Base Corrector (For-A Corporation, Japan), and the video image was processed by Image-1 (Universal Imaging Corporation, Media, Pennsylvania) hardware and software installed in an IBM 486-compatible computer.

Platelet preparation. Blood samples were obtained from adult mongrel dogs by venipuncture using 3.8% trisodium citrate as the anticoagulant (1 volume citrate to 9 volumes blood). Platelet-rich plasma (PRP) was prepared by centrif-

ugation of whole blood at 165 g for ten min. Platelet-rich plasma was removed with a polyethylene pipette, and platelets were separated from plasma proteins by passage through a Sepharose CL-4B column having a 40-ml total bead volume. The column was equilibrated with calcium-free Tyrodes' buffer, pH 7.4 (136 mM NaCl, 2.7 mM KCl, 0.42 mM NaH_2PO_4, 12 mM $NaHCO_3$, 1 mM $MgCl_2$, 1 g/l dextrose, 2 g/l albumin).

Preparation of pS-NO-BSA. Bovine serum albumin covalently modified to carry multiple S-NO functional groups was synthesized as described by Stamler et al. (9). Fatty acid-free bovine serum albumin (200 mg/ml) was exposed to a 1.4-molar fold excess of $NaNO_2$ in 0.5 N HCL for 30 min at room temperature and neutralized with an equal volume of Tris-buffered saline (10 mM Tris [hydroxymethyl] aminoethane, pH 7.4 and 150 mM NaCl) and of 0.5 N NaOH. Bovine serum albumin covalently modified to carry multiple S-NO functional groups was prepared as previously described and kindly provided by NitroMed, Inc. (Boston, Massachusetts) (10). The preparation used contained eight moles of NO per mole of albumin.

Platelet surface-activation on collagen. Collagen is a major constituent of the subendothelial blood vessel wall and was used to provide a thrombogenic substrate similar to that of an injured vessel. The bottom (24 × 50 mm) glass cover slip of the parallel plate chamber was covered with collagen solution (type I fibrillar collagen from bovine achilles tendon, Sigma Chemical Co., St. Louis, Missouri). A pipette tip was used to spread 10 μl of the 25% collagen solution on three cover slips. The coated slide was set aside for 45 to 60 min to dry and polymerize. One collagen-coated cover slip was then coated with a 5% solution of BSA (20 μl). Another collagen-coated cover slip was coated with 0.5 mM of pS-NO-BSA (20 μl). The third collagen-coated cover slip was used as a control. After assembling each chamber, 50 μl of platelet suspension was allowed to fill each chamber by capillary action. Each chamber was placed on an inverted-stage microscope (Nikon, DIAPHOT-TMD, Torrance, California). The microscope was focused on the bottom surface of the chamber to visualize platelet attachment and spreading over time.

Platelet surface-activation on glass. Before assembling the parallel-plate chambers, the lower (24 × 50 mm) glass cover slip was coated with a 5% solution of BSA (20 μl) and another glass cover slip was coated with 0.5 mM NO of pS-NO-BSA (20 μl). A third glass cover slip was used as a control. After assembling each chamber, a 50 μl droplet of platelet suspension was allowed to fill each chamber by capillary action. Each chamber was placed on an inverted-stage microscope (Nikon, DIAPHOT-TMD) to visualize platelet attachment and spreading on the surface over time.

In vitro effect of a pS-NO-BSA coated versus uncoated Palmaz-Schatz (P-S) stent on platelet cGMP levels. Twelve P-S stents were immersed in 1 N HCl for one min then rinsed in distilled water and allowed to dry. They were then dip-coated in 800 to 1,000 μM pS-NO-BSA three times for ten min, followed by ten min of air drying. Five to seven days later, three coated P-S stents and a control uncoated stent were rinsed with distilled water and dried. Each stent was suspended in a polypropylene test tube containing five ml aliquots of porcine platelet-rich plasma (PRP) and 100 μM isobutylmethylxanthine for two min. At the end of the two min periods, the stents were removed and 5 ml of 10% cold trichloroacetic acid (TCA) was immediately added to the PRP. The mixture of PRP and TCA was then centrifuged and the supernatant frozen at −70°C for later analysis of platelet cGMP content.

In vivo effect of a pS-NO-BSA coated versus uncoated Palmaz-Schatz stent on the deposition of [^{111}In]-labeled platelets. Six pigs (16–18 kg) were anesthetized with ten mg/kg ketamine and five mg acepromazine IM after which 20 mg/kg sodium pentobarbital was administered IV. Fifty milliliters of blood was removed from the pig and autologous platelets were labeled with [^{111}In]-oxine (15). The femoral artery and vein were cannulated for arterial pressure measurement and infusion of the [^{111}In]-labeled platelets. The right and left carotid arteries were dissected out, and an electromagnetic flow probe was placed proximally on each artery. Blood flow was continuously monitored. The autologous [^{111}In]-labeled platelets were reinjected 30 min prior to deploying a coated and an uncoated P-S stent in the same animal. Stents were dip coated as previously described. An uncoated and a coated P-S stent were placed in the right or left carotid arteries, respectively, under fluoroscopic guidance using a Sci-Med PTCA NC Cobra 3.0 mm dilation catheter (Scimed Life Systems Inc., Maple Grove, Minnesota). The balloon was inflated to 12 atmospheres for 60 s to deploy the stents with a repeat inflation after one min for 60 s. The stents were then exposed to flowing blood for two h. At the end of 2 h of blood perfusion, the pigs were sacrificed with an overdose of pentobarbital, and the vessels were harvested and fixed in formaldehyde. The stented portions of the arteries were placed in the well of an NaI counter to determine radio-labeled platelet accumulation on the stent surface. The investigation conforms with the guidelines for the care and use of laboratory animals by the US National Institute of Health (NIH publication 1996) and the University of Wisconsin Research Animal Resource Center.

Statistics. All data values are reported as mean ± standard deviation. The statistical significance of the difference between measurements is obtained from the paired student *t* test and is reported by the p value.

RESULTS

pS-NO-BSA coating of glass. Figure 3 shows photomicrographs of platelet adhesion on glass at ten min, BSA coated glass at 30 min and pS-NO-BSA-coated glass at 30 and 60 min after the platelet suspension was placed on the

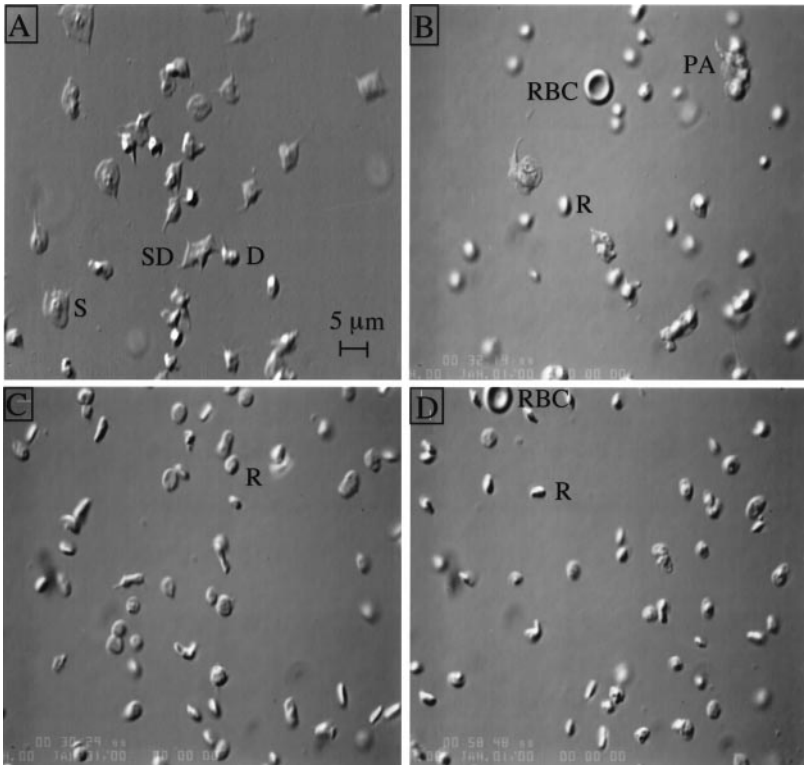

Figure 3. Photomicrographs of platelet adhesion on **(A)** glass at 10 min, **(B)** BSA-coated glass at 30 min, pS-NO-BSA coated glass at **(C)** 30 min and **(D)** 60 min after the platelet suspension was placed in the parallel plate chamber. At 10 min all the platelets have attached to the glass surface. At 30 min many platelets have attached to the BSA-coated glass and some platelet aggregates formed, while at 30 and 60 min most of the platelets were still unattached to the pS-NO-BSA-coated collagen. R = platelet is round and unattached; D = platelet is in dendritic stage with the dendrites attached to the surface; SD = platelet is in spread dendritic stage; S = platelet is fully spread over the surface; PA = platelet aggregates; RBC = red blood cell.

surface. At ten min, all platelets attached to the glass surface. At 30 min many platelets attached to the BSA-coated glass and some platelet aggregates formed, while at 30 and 60 min most of the platelets were still unattached to the pS-NO-BSA-coated glass. The platelets that attached to the glass and BSA-coated glass surfaces quickly extended their pseudopods, spread over the surface and caused other platelets to aggregate. However, the few platelets that attached to the pS-NO-BSA-coated surface did not spread over the surface. Figure 4 shows the percent of platelets that attached to glass, BSA-coated glass and pS-NO-BSA-coated glass during a 50 min period (the data points represent averages from four experiments). After 50 min 100% of the platelets attached to the glass surface, and 40 ± 15% of the platelets attached to the BSA-coated glass surface. However, at 50 min, only 14 ± 6% of the platelets attached to the pS-NO-BSA-coated glass surface with the remaining platelets freely moving (indicating no attachment or adhesion). This suggests that coating the glass surface with pS-NO-BSA makes it less thrombogenic than BSA-coated glass.

pS-NO-BSA coating of collagen. Similar results were observed for platelet adhesion on collagen, BSA-coated collagen and pS-NO-BSA-coated collagen. At 30 min, most of the platelets attached to the BSA-coated collagen while at 60 min most of the platelets were still unattached to the pS-NO-BSA-coated collagen. The platelets that attached to collagen and BSA-coated collagen have extended their pseudopodia along the collagen fibers and spread over the surface. However, the few platelets that attached to the pS-NO-BSA-coated collagen did not spread. Figure 5 shows the percentage of platelets that attached to collagen,

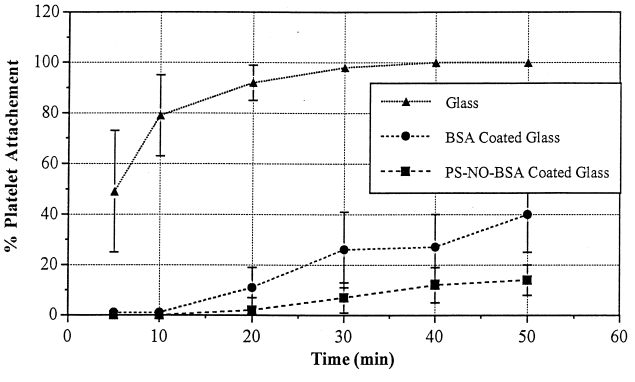

Figure 4. Percent platelet attachment on glass, BSA-coated glass and pS-NO-BSA-coated glass as a factor of time of platelet exposure to the surface (n = 4).

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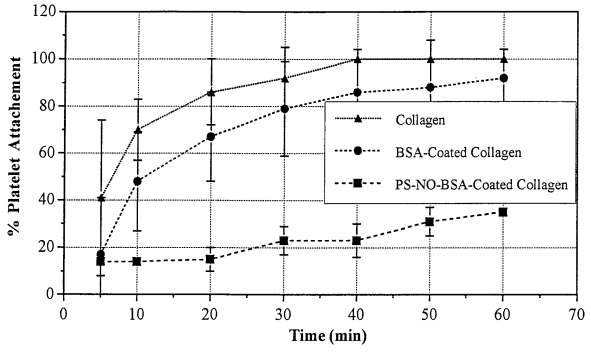

Figure 5. Percent platelet attachment to collagen type I, BSA-coated collagen and pS-NO-BSA coated collagen as a function of time (n = 4).

BSA-coated collagen and pS-NO-BSA-coated collagen during a 60 min period (the data points are averages from four experiments). After 30 min, 92 ± 13% of the platelets were attached to the collagen surface, and 79 ± 20% of the platelets were attached to the BSA-coated surface. However, for the pS-NO-BSA-coated surface at 60 min, only 35 ± 24% of the platelets were attached to the collagen fibers with the remaining platelets floating in the buffered platelet suspension. This observation demonstrates that coating the collagen surface with pS-NO-BSA renders it significantly less thrombogenic compared with BSA-coated collagen.

Effect of a pS-NO-BSA coating of P-S stents on platelet cGMP. The average cGMP in the PRP exposed to pS-NO-BSA coated stents was 5.9 ± 0.7 pmoles cGMP/10^8 platelets, compared with 2.7 ± 0.9 pmoles cGMP/10^8 platelets ($p < 0.01$, n = 12) for uncoated stents (Fig. 6). This observation suggests that the pS-NO-BSA coating did adhere to the P-S stents and that the NO was available to dissociate from the coated stents into the platelet rich plasma and increase platelet cGMP content.

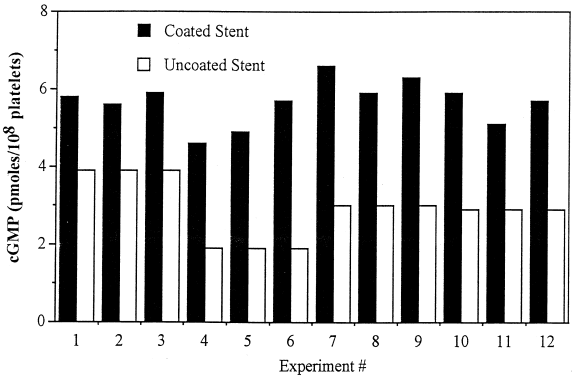

Figure 6. Cyclic GMP contents of platelets incubated with pS-NO-BSA-coated and uncoated stents for 2 min in platelet rich plasma. The average platelet cGMP was 2.9 ± 0.7 p moles/10^8 platelets for the uncoated stents and 5.9 ± 0.7 p moles/10^8 platelets for the pS-NO-BSA-coated stents ($p < 0.01$, n = 12).

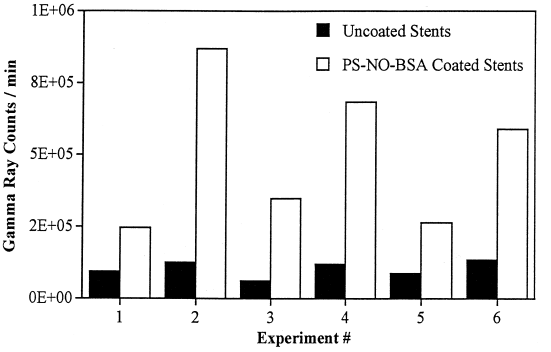

Figure 7. Platelet accumulation (proportional to gamma counts) on pS-NO-BSA-coated and uncoated Palmaz-Schatz stents deployed in the carotid arteries of pigs for 2 h. Platelet accumulation on the uncoated stents was 4.5 ± 1.6 fold greater than that on the pS-NO-BSA-coated stents ($p < 0.01$, n = 6).

In vivo effect of a pS-NO-BSA coating of P-S stents on the deposition of [^{111}In]-labeled platelets. Figure 7 shows the gamma counts detected from radiolabeled platelets that attached to the coated and uncoated stents after being deployed in the carotid arteries of pigs for 2 h. The average counts were 91,000 ± 42,000/min for the coated stents and 435,000 ± 290,000/min for the uncoated stents. The average platelet accumulation on the uncoated stents after implantation in pigs with circulating [^{111}In]-labeled platelets was 4.5 ± 1.6-fold higher than the platelet accumulation on the pS-NO-BSA-coated stents (n = 6, $p < 0.01$).

DISCUSSION

These data suggest that the pS-NO-BSA coating was significantly more effective than BSA alone in inhibiting platelet surface activation, adhesion and aggregation on collagen-coated and glass surfaces. Fifty min after exposure, all the platelets were attached to the glass and collagen surfaces. With the albumin coating, most of the platelets were attached, but with the pS-NO-BSA coating, most of the platelets were still freely moving on the glass and collagen surfaces, indicating that they were not surface activated. This study also demonstrates that a pS-NO-BSA coating adheres to the P-S stent and that the NO is able to dissociate from the stent to increase platelet cGMP content. In addition, this coating significantly reduces the deposition of autologous [^{111}In]-labeled platelets on stents placed in pig carotid arteries for two h compared with uncoated stents placed in pig carotid arteries. This effect is likely due to the antiadhesive effect of albumin combined with the direct antiadhesive and antiaggregation effect of NO.

Coating of damaged arterial surfaces with plasma proteins. Artificial surfaces in contact with blood quickly develop a coating of adsorbed plasma protein that governs subsequent interaction with blood cells. This initial protein adsorption occurs within seconds, before the blood platelets

and other cellular components reach the surface (16,17). Much research has focused on the role of the most prevalent plasma proteins. Albumin is by far the most abundant protein in serum, and albumin coating has been used to make synthetic surfaces more thromboresistant (18–20). By contrast, polymer surfaces coated with fibrinogen, another abundant protein in serum, promotes platelet activation and presents a very thrombogenic surface (16). Comparison of the results of sequential protein adsorption to those of competitive adsorption from an albumin-fibrinogen mixture suggests that fibrinogen has a higher binding affinity for commonly used biopolymer surfaces (16). The initial protein layer adsorbed to the polymer surface is very important in determining the thrombogenicity of the surface (17). Precoating the synthetic surface with albumin renders the surface less thrombogenic and more blood compatible (16). In our experiments, coating the glass, collagen and stent surfaces with nitrosated albumin provided a much more thromboresistant surface than did coating with albumin alone. This effect is likely a direct consequence of the antiplatelet effects of NO.

Mechanisms of platelet inhibition by NO. Both endogenous and exogenous NO or NO-donating compounds inhibit platelet adhesion (21,22) and aggregation (23–25). Furthermore, NO generating compounds potentiate the activity of thrombolytic factors to provide further protection against vascular occlusion (26). The inhibitory response of platelets is mediated by NO binding to the heme iron of soluble guanylyl cyclase (GC) which activates the enzyme and leads to the conversion of magnesium guanosine 5′-triphosphate to guanosine 3′,5′-monophosphate (cyclic GMP) (27–29). cGMP regulates receptor-mediated Ca^{2+} influx and mobilization in platelets which are necessary for platelet activation. The increase in cGMP reduces the platelet cytosolic calcium concentration (30). It appears that cGMP is a more potent inhibitor of Ca^{2+} influx than of Ca^{2+} mobilization in platelets (31).

Among the most stable NO donating compounds are the S-nitroso-thiols such as S-nitrosated albumin (9). S-Nitroso-thiols serve as carriers in the mechanism of action of EDRF by stabilizing the labile NO (9). In the dog, nitrosated albumin has been shown to inhibit ex vivo platelet aggregation and significantly prolongs the template bleeding time (32). Normal endothelial cells are able to synthesize NO which accounts for the biological properties of endothelium-derived relaxing factor (EDRF) (33). Nitric oxide released luminally inhibits the interaction of circulating platelets with the damaged vessel wall. The capacity of the endothelium to synthesize NO is reduced in human coronary atherosclerosis (34). The NO inhibitory effect of platelets is lost in chronic atherosclerotic lesions and in acutely induced lesions, such as those caused by balloon angioplasty. Collagen is a major constituent of the subendothelial blood vessel wall, and collagen type I predominates in atherosclerotic arterial subendothelium, presenting a very thrombogenic surface (35). In vitro experiments of flow in a parallel plate chamber have shown that platelet adhesion is the result of the adsorption of large vWF multimers onto collagen and the subsequent binding of platelet GPIb to the insolubilized vWF (36).

Summary. In conclusion, the S-nitrosated albumin coating was significantly more potent than an albumin coating in inhibiting platelet surface activation, adhesion and aggregation on collagen-coated and glass surfaces. The coating was also avid for metal stent surfaces and significantly reduced platelet attachment to the surface during an initial 2 h perfusion period in vivo. This result is probably due to the antiadhesive properties of albumin combined with the antiplatelet effects of NO. By preventing early platelet attachment and activation to the thrombogenic surface, an S-nitrosated albumin coating may reduce the incidence of acute thrombosis and restenosis.

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REFERENCES

1. Myers PR, Minor RL, Guerra R Jr, Bates N, Harrison DG. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resembles S-nitrosocysteine than nitric oxide. Nature 1990;345:161–3.
2. Radomski MW, Palmer RM, Moncada S. Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. Br J Pharmacol 1987;92:181–7.
3. Stamler JS, Loscalzo J. The antiplatelet effects of organic nitrates and related nitroso-compounds in vitro and in vivo and their relevance to cardiovascular disorders. J Am Coll Cardiol 1991;18:1529–36.
4. Folts JD, Stamler JS, Loscalzo J. Intravenous nitroglycerin infusion inhibits cyclic blood flow responses caused by periodic platelet thrombus formation in stenosed dog coronary arteries. Circulation 1991;83:2122–7.
5. Rovin JD, Stamler JS, Loscalzo J, Folts JD. Sodium nitroprusside, an endothelium relaxing factor congener, increases platelet cyclic GMP levels and inhibits epinephrine-exacerbated in vivo platelet thrombus formation in stenosed canine coronary arteries. J Cardiovasc Pharmac 1993;22:626–31.
6. Garg UC, Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat smooth muscle cells. J Clin Invest 1989;83:1774–7.
7. Amiji M, Park H, Park K. Study on the prevention of surface induced platelet activation by albumin coating. J Biomater Sci Polymer 1992;3:375–88.
8. Riccitelli SD, Schlatterer RG, Hendrix JA, Williams GB, Eberhart RC. Albumin coatings resistant to shear-induced desorption. Trans Am Soc Artif Intern Organs 1985;31:250–6.
9. Stamler JS, Simon DI, Osborne JA, et al. S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. Proc Natl Acad Sci 1992;89:444–8.

10. Marks DS, Vita JA, Folts JD, Keaney JF Jr, Welch GN, Loscalzo J. Inhibition of neointimal proliferation in rabbits after vascular injury by a single treatment with a protein adduct of nitric oxide. J Clin Invest 1995;96:2630–8.
11. Folts JD. Coating Palmaz-Schatz stents with a unique NO donor reduces the need for post-procedure anticoagulation when placed in pig carotid arteries. J Invest Med 1995;43:476A.
12. Folts JD, Keaney JF, Loscalzo J. Local delivery of nitrosated albumin to stenosed and damaged coronary arteries inhibits platelet deposition and thrombosis [special edition]. Jam Col Card 1995;377A.
13. Albrecht RM, Goodman SL, Simmons SR. Distribution and movement of membrane-associated platelet glycoproteins: use of colloidal gold with correlative video-enhanced light microscopy, low-voltage high-resolution scanning electron microscopy, and high-voltage transmission electron microscopy. Amer J Anat 1989;185:149–64.
14. Olorundare OE, Simmons SR, Albrecht RM. Cytochalasin D and E: effects on fibrinogen receptor movement and cytoskeletal reorganization in fully spread, surface-activated platelets: a correlative light and electron microscopic investigation. Blood 1992;79:99–109.
15. Heyns AP. Method for labeling platelets with In111-oxine. In: Heyns AP, Badenhorst PN, Lotter MG, editors. Platelet Kinetics and Imaging, VI. Boca Raton, Florida: CRC Press, 1985:153–7.
16. Park K, Mosher DF, Cooper S. Acute surface-induced thrombosis in the canine ex vivo model: importance of protein composition of the initial monolayer and platelet activation. J Biom Mat Res 1986;20:589–612.
17. Brash JL. Protein interactions with artificial surfaces. In: Salzman EW, editor. Interaction of the Blood With Natural and Artificial Surfaces. New York: Marcel Dekker, Inc., 1981:37.
18. Brynda E, Houska M, Pokorrna Z, Cepalova NA, Moiseev YV, Kalal J. Irreversible adsorption of human serum albumin onto polyethylene film. J Bioeng 1978;2:411–8.
19. Ihlenfeld JV, Cooper SL. Transient in vivo protein adsorption onto polymeric biomaterials. J Biomed Materials Res 1979; 13:577–91.
20. Ishikawa Y, Sasakawa S, Takase M, Osada Y. Effective albumin immobilization by plasma polymerization on platelet reactivity. Thromb Res 1984;35:193–202.
21. Groves PH, Penny WJ, Cheadle HA, Lewis MJ. Exogenous nitric oxide inhibits in vivo platelet adhesion following balloon angioplasty. Cardio Res 1992;26:615–9.
22. Radomski MW, Palmer RM, Moncada S. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. Lancet 1987;2:1057–8.
23. Lam JY, Chesebro JH, Fuster V. Platelets, vasoconstriction and nitroglycerin during arterial wall injury. A new antithrombotic role of an old drug. Circulation 1988;78:712–6.
24. DeCaterin R, Giannessi D, Bernini W, Lazzerini G, Mazzone A, Lombardi M. In vivo actions of organic nitrates on platelet function in humans. Z Kardiol 1989;78 Suppl 2:56–60.
25. Azuma H, Ishikawa M, Sekizaki S. Endothelium-dependent inhibition of platelet aggregation. Br J Pharmacol 1986;88: 411–5.
26. Korbut R, Lidbury PS, Vane JR. Prolongation of fibrinolytic activity of tissue plasminogen activator by nitrovasodialators. Lancet 1990;335:669.
27. Loscalzo J. N-Acetylcysteine potentiates inhibition of platelet aggregation by nitroglycerin. J Clin Invest 1985;76:703–8.
28. Keaney JF Jr, Simon DI, Stamler JS, et al. NO forms an adduct with serum albumin that has endothelium-derived relaxing factor-like properties. J Clin Invest 1993;91:1582–9.
29. Mellion BT, Ignarro LJ, Ohlstein EH, Pontecorvo EG, Hyman AL, Kadowitz PJ. Evidence for the inhibitory role of guanosine 3′,5′-monophosphate in ADP-induced human platelet aggregation in the presence of nitric oxide and related vasodilators. Blood 1981;57:946–55.
30. Nakashima S, Tohmatsu T, Hattori H, Okano Y, Nozawa Y. Inhibitory action of cyclic GMP on secretion, polyphosphoinositide hydrolysis and calcium mobilization in thrombin stimulated human platelet. Biochem Biophys Res Commun 1986;135:1099–104.
31. Morgan RO, Newby AC. Nitroprusside differentially inhibits ADP-stimulated calcium influx and mobilization in human platelets. Biochem J 1989;258:447–54.
32. Keany JF, Stamler JS, Scharfstein J, Folts JD, Loscalzo J. NO forms a stable adduct with serum albumin that has potent antiplatelet properties in vivo. Clin Res 1992;40:194A.
33. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988; 333:664–6.
34. Chester AH, O'Neil GS, Moncada S, Tadjkarimi S, Yacoub MH. Low basal and stimulated release of nitric oxide in atherosclerotic epicardial coronary arteries. Lancet 1990;336: 897–900.
35. Barnes MJ. Collagen in atherosclerosis. Collagen Rel Res 1985;5:65–97.
36. Alevriadou BR, Moake JL, Turner NA, et al. Real-time analysis of shear-dependent thrombus formation and its blockade by inhibitors of vonWillebrand factor binding to platelets. Blood 1993;81:1263–76.